Remarks

Claims 2, 3, and 5-11 are pending in the present application. Claims 12, 13, and 14 are newly added claims. Claim 7 has been cancelled herein without prejudice to the filing of a continuation application. Claims 5, 8, 9, and 10 have been amended herein. Claim 5 has been amended by removing an element of the claim. Support for amended claim 8 is found at page 8, lines 27-29 of the specification as filed. Support for amended claim 9 is found at page 7, lines 9-12, and in FIGS. 1 and 2 of the specification. Support for amended claim 10 is found in the specification at page 7, lines 9-12. Support for newly added claims 12, 13, and 14 is found throughout the specification as filed as more fully discussed below.

Request for Continued Examination

This response is being submitted with a Request for Continued Examination under 37 CFR 1.114.

Benefit of Priority

The Examiner, at page 2 of the Office Action, has again denied benefit of priority of the filing date of U.S. Provisional Application No. 60/188,391, filed March 10, 2000, for independent claims 2, 3, and 5, and dependent claims 6, 7, 8, 9, 10, and 11. The Examiner now alleges that the term "inhibit," is broader than the term "prevent," which was used in the '391 provisional application, and that the terms are not interchangeable.

The Examiner again alleges that the hematopoietic stem cell separation step of independent claim 5 of the present application is not disclosed in '391. The Examiner further alleges at page 2 of the Office Action that the '391 application indicates a separation step involving peripheral blood mononuclear cells (PBMC), whereas claim 5 of the present application recites a step involving hematopoietic stem cells. Thus, the Examiner asserts that fractions of CD34+ and CD34- cells of the '391 application are not the same as the cell fractions of the instant claims.

Applicants respectfully disagree for the following reasons.

Because there is no art relevant to the priority date, and thus no art requiring a determination of priority, any question as to benefit of priority is believed moot. However,

denial of the benefit of priority is addressed below to ensure that every issue raised in the Office Action is addressed.

Applicants assert that, contrary to the Examiner's allegation that "inhibit" has a broader meaning than "prevent" because it is defined as "retard or prevent," the definition should be interpreted to mean that the terms are interchangeable based on the context in which they are used. Both terms are used throughout the pending specification as filed, and the term "prevent" was used throughout provisional application '391. For example, in the present application, "prevent" is used at page 2, line 18, page 3, line 1, and at page 4, lines 19 and 20, while the term "inhibit," is used at page 1, line 24, page 5, lines 11-26, and in original claims 1 and 5. Thus, Applicant submits that recitation of the term "inhibiting" in independent claims 2, 3, and 5, as well in dependent claims 6, 8, 9, 10, and 11 should not be denied the benefit of priority of the '391 provisional application.

Applicants respectfully submit that the separation step of claim 5 of the present application is disclosed in the '391 provisional application. Claim 5 recites "separating hematopoietic stem cells" to obtain fractions of CD34+ and CD34- cells. It was known to those of ordinary skill in the art at the time the application was filed, that peripheral blood mononuclear cells (PBMCs) are a source of hemopoietic stem cells, including CD34+ and CD34- cells. For example, see the abstract of "Phenotypic differences of CD34-positive stem cells harvested from peripheral blood and bone marrow obtained before and after peripheral blood stem cell collection" (Inaba et al., 1994, Bone Marrow Transplant. 13:5:527-532; provided herewith) and the abstract of "Harvesting, characterization, and culture of CD34+ cells from human bone marrow, peripheral blood, and cord blood" (Van Epps et al., 1994, Blood Cells, 20:411-423; provided herewith). At page 2, lines 8-12 of '391 it is stated:

> Another aspect of the present invention is a method of preventing GVHD in a mammal requiring transplant of $CD34^+$ stem cells, comprising administration of a therapeutically effective amount of LLME to $CD34^-$ PBMC ex vivo prior to co-administration of said $CD34^-$ PBMC treated with LLME and $CD34^+$ stem cells in said mammal requiring transplant of $CD34^+$ stem cells.

Thus, '391 describes the use of two different populations of cells derived from a population of hematopoietic stem cells (HSC), namely from PBMCs. Although '391 does not specifically recite the act of "separating" the cells, the act is implied in using the two

populations or fractions of cells, and one of ordinary skill in the art would understand that the act of separating the cells would be required to obtain the two fractions of cells from the parent HSC population. Otherwise, it would not be possible to perform the step of ". . . co-administration of said $CD34^-$ PBMC treated with LLME and $CD34^+$ stem cells in said mammal requiring transplant of $CD34^+$ stem cells," recited in '391, because the $CD34^-$ cell population is treated with LLME and the $CD34^+$ stem cells are not. Further support is found in claim 3 of '391, which recites co-administration of the two populations of cells as well. Therefore, the use of the two populations of cells is fully disclosed in the parent '391 application. Thus, Applicant submits that claim 5, and its dependent claims 6, 8, 9, 10, and 11, should not be denied benefit of priority of application '391.

In view of the present specification, the context in which the cell populations are described, and the prior art usage as discussed above, one of ordinary skill in the art would readily understand that the scope of stating that the two population of cells, wherein one population is treated and the other is not, would require separating those cells from a heterogeneous cell population source of cells, which source could be PBMCs or another source of hematopoietic stem cells.

Although not necessarily agreeing with the reasoning of the Examiner that the term "inhibit" has a meaning which is broader than the term "prevent," Applicants have, however, added new independent claims, 12, 13, and 14, which recite the term "prevent," as used in provisional application '391. Support for reciting the term "preventing" in the newly added claims is found throughout the present application as filed, particularly at page 2, line 18, page 3, line 1, and at page 4, lines 19 and 20. For example, the role of L-leucyl-L-leucine methyl ester (LLME) in preventing graft versus host disease (GVHD) in vivo is discussed at page 4, lines 19 to 24, of the present application, where it is stated that "LLME has demonstrated salutary effects in preventing GVHD in animal models. Ex Vivo treatment of bone marrow grafts with LLME can completely prevent GVHD . . ." Support for adding claims reciting "preventing" is also found at page 2 of the Office Action mailed March 27, 2003, where the Examiner asserts that the term "prevent" is encompassed by the broader term "inhibit."

Based on the details outlined above, Applicants respectfully submit that the instant application does not differ significantly from '391, and request that the denial of benefit of

priority to '391 be withdrawn as to independent claims 2, 3, and 5, dependent claims 6, 8, 9, 10, and 11. Applicant further asserts that newly added claims 12, 13, and 14 should receive benefit of priority for the reasons discussed above.

Response to the 35 U.S.C. § 103(a) rejection

Claims 2, 3, 5, and 6-11 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Rosenfeld in view of Small et al. (Blood, 1999, 93:467-480) and U.S. Patent No. 5,668,112 to Lipsky et al.

In the view of the Examiner, Rosenfeld teaches a method of inhibiting GVHD in a human requiring donor lymphocyte infusion, comprising contacting donor lymphocytes to be infused with a therapeutically effective amount of LLME ex vivo and infusing said cells into the human and inhibiting GVHD. The Examiner also asserts that the limitation of claim 1, "a mammal in need of DLI," is met because the patients had received total body irradiation and would die absent an infusion of donor lymphocytes. It is also the view of the Examiner that Rosenfeld teaches that LLME treatment causes a reduction in the number of human progenitor cells and CFU-GM. The Examiner also asserts that Rosenfeld teaches LLME treatment of at least 1 micromolar, from about 10 to about 500 micromolar, and at least about 500 micromolar. The Examiner further asserts that Rosenfeld teaches treatment with LLME for at least about 15 minutes and the freezing of treated cells.

The Examiner alleges that Small et al. teaches donor lymphocyte infusion post-stem cell transplantation to prevent viral infections and that the risk of opportunistic infection in stem cell transplant patients is inversely proportional to CD4 T cell counts.

The Examiner also alleges, at page 4 of the Office Action, that the '112 patent of Lipsky et al. teaches that NK cells and cytotoxic T cells are primarily responsible for GVHD after donor lymphocyte infusion (DLI) and the ex vivo treatment with LLME can be used to selectively kill NK and cytotoxic T cells before DLI.

The Examiner asserts at page 4 of the Office Action that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to infuse LLME-treated donor lymphocytes as taught by Rosenfeld, into a mammal after stem cell transplantation, in view of the combined teachings of Small and the '112 patent. The Examiner further asserts that one of ordinary skill in the art at the time the invention was

made would have been motivated to use LLME-treated donor lymphocytes (as opposed to untreated lymphocytes) for DLI, because in the view of the Examiner the DLI would have been increased in CD4 cells, and that in particular, the increase would have been achieved without an additional increase in the depleted CD8+ and NK cells, and would thus, have increased the ability to inhibit opportunistic infection without increasing the risk of GVHD.

The Examiner then rejects claim 5, alleging that it would have been obvious in view of the combined references to separate cells for transplantation into CD34+ (stem cell) and CD34- (nonstem cell) fractions before LLME treatment, and treat only the CD34- fraction (comprising NK and cytotoxic T cells), while leaving the CD34+ fraction (CFU-GM containing) untreated, thus obtaining the benefits of NK and cytotoxic T cell reduction, without concurrent CFU-GM reduction.

Applicants respectfully submit that the combination of Rosenfeld, Small, and '112 does not render claims 2, 3, 5, 6, and 8-11, nor new claims 12, 13, and 14, *prima facie* obvious under 35 U.S.C. § 103(a), claim 7 having been cancelled herein, for the following reasons.

Preliminarily, the three-prong test which must be met for a reference or a combination of references to establish a prima facie case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

To support a case of *prima facie* obviousness, a combination of references must: (1) suggest to those of ordinary skill in the art that they should make the claimed invention, and (2) reveal to those of ordinary skill in the art that they would have a reasonable expectation of success. In re Vaeck, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be found in the prior art and not in Applicant's disclosure. In re Dow Chemical Company, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). None of these criteria have been met here.

Claim 2 recites a method of inhibiting GVHD in a mammal requiring DLI infusion in which allogeneic T cell-depleted hematopoietic stem cell transplantation occurs before donor lymphocytes are infused. Claim 3 recites a method of inhibiting GVHD in a mammal requiring DLI infusion in which donor engraftment with hematopoietic stem cells occurs before LLME-treated donor lymphocytes are infused. Claim 5 recites a method of inhibiting GVHD wherein hematopoietic stem cells are separated into CD34+ and CD34- fractions, the CD34- cells are treated with LLME to eliminate cytotoxic T cells, and then the two fractions are co-administered.

Rosenfeld, combined with Small and '112, does not teach or suggest all of the claimed elements of the present invention. First, Rosenfeld teaches treating bone marrow with LLME to eliminate cytotoxic T cells (see abstract) prior to transplantation and does not teach or suggest treating donor lymphocytes with LLME for infusion after allogeneic T cell-depleted hematopoietic stem cell transplantation, as claimed in independent claims 2, 3, or 5, or new independent claims 12, 13, and 14. Furthermore, as admitted by the Examiner, Rosenfeld does not teach the use of LLME-treated DLI after hematopoietic stem cell transplantation or engraftment or the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, as recited in independent claims 2, 3, 5, 12, 13, and 14 of the present application.

In addition, treatment of marrow with LLME at concentrations of 375 and 500 micromolar by Rosenfeld caused high levels of toxicity to stem cells (see Table 2 and page 681, column 2, last paragraph, bridging page 682). The present method spares treating the marrow and allows higher concentrations of LLME to be used because a select population of cells is being treated. Thus, Rosenfeld teaches away from using high doses of LLME as recited in claim 9, e.g., 375 micromolar, of the present application, because their procedure causes stem cell toxicity.

The patients in Rosenfeld do not meet the limitation of claim 1, "a mammal in need of DLI," just because they had received total body irradiation. Subsequent to irradiation, the patients in Rosenfeld received only a bone marrow transplant, not DLI. Thus, the patients did not need DLI. Rosenfeld illustrates the typical clinical practice, which is to treat patients with only a bone marrow transplant, and not to follow the transplant with DLI.

Contrary to the assertions of the Examiner, Rosenfeld does not teach the use of at least 1 micromolar LLME, nor does it teach from about 10 to about 500 micromolar, or at least about 500 micromolar LLME. Rosenfeld treated bone marrow with only three different concentrations of LLME (250, 375, or 500 micromolar; see abstract and Tables 2-4), and in fact showed that concentrations of 375 and 500 micromolar LLME were toxic to the treated bone marrow (Table 2). Rosenfeld does not teach the use of LLME at about 1 to about 250 micromolar to treat donor lymphocytes or CD34- cells as in amended claim 8, nor does Rodenfeld teach the use of 375 micromolar to treat donor lymphocytes or CD34- cells as in amended claim 9.

Rosenfeld teaches agitating bone marrow cells in the presence of LLME for 15 minutes, but does not teach contacting donor lymphocytes or a CD34- hematopoietic stem cell fraction for 15 minutes with LLME, as recited in amended claim 10 of the present application. Contrary to the Examiner's assertion that Rosenfeld teaches cryopreserving LLME treated cells as recited in claim 11, Rosenfeld only teaches cryopreserving untreated bone marrow cells. The last sentence at page 679, column 2, bridging page 680 column 1, states "An attempt was made to harvest 6 x 10^8 nucleated bone marrow cells/kg so that 4 x 10^8/kg could be treated with LLME and 2 x 10^8/kg could be cryopreserved in case of graft failure." Thus, Rosenfeld does not even teach cryopreserving LLME treated bone marrow cells, much less cryopreserving LLME treated DLI or CD34- cell fractions. Therefore, Rosenfeld does not teach or suggest the elements of dependent claims 8, 9, 10, and 11 of the present application.

At page 679, in the sentence bridging columns 1 and 2, Rosenfeld states "However, LLME treatment of marrow did not prevent lethal GVHD in transplants with only class 2 discrepancies or with class 1 and class 2 with many non-MHC differences," citing Thiele et al., 1988, J. Immunol. 141:3377 and Blazar et al., 1991, Blood 78:3093. It is known that the development of GVHD related to class 2 differences is mediated by CD4+ T cells (Korngold et al., 1985, J. Immunol. 135:3004-3010; Sprent et al., 1986, J. Exp. Med. 163:998-1011; copies of which are supplied herewith). Thus, based on the teachings of Rosenfeld, Korngold and Sprent, LLME treatment of donor lymphocytes would not be expected to prevent GVHD, due to the high percentage of CD4+ T cells present in the infused cells. Rosenfeld's teachings further suggest that infusing donor lymphocytes treated with LLME

would not be advisable because both CD4+ and CD8+ GVHD responses could be involved. Therefore, Rosenfeld teaches away from infusing LLME treated donor lymphocytes to inhibit GVHD as claimed in claims 2, 3 and 5, or to prevent GVHD as recited in newly added claims 12, 13, and 14.

Small does not remedy the deficiencies of Rosenfeld, in that it does not teach or suggest the use of LLME to prepare T cell-depleted DLI for use in inhibiting or preventing GVHD following hematopoietic stem cell transplantation, nor does Small teach the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, as claimed in claims 5 and 14 of the present application. Small only teaches using unirradiated whole populations of donor lymphocytes, e.g., lymphocytes which have not been treated with LLME or manipulated in any way. Furthermore, Small teaches administering the donor lymphocytes solely based on the $CD3^+$ cell count. In fact, Small does not teach the use of LLME.

In addition, '112 does not remedy the deficiencies of Rosenfeld and Small. This reference teaches treating whole marrow with LLME prior to transplantation (column 4, lines 38-58; column 5, lines 4-9; column 6, lines 60-67), and does not teach treating donor lymphocytes for use after transplantation or separating $CD34^+$ cells from $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, as claimed in claims 5 and 14 of the present application.

Contrary to the assertion of the Examiner, the '112 patent does not teach that NK cells and cytotoxic cells are primarily responsible for GVHD after DLI. Instead, '112 teaches that "[s]ince cytotoxic T cells (CTL) derived from donor bone marrow appear to be the final mediators of GVHD, in vitro treatment of donor bone marrow with an agent which selectively damages cytotoxic T cell precursors is also likely to be of benefit," (column 9, lines 17-21). In fact, '112 teaches that the patients themselves should be treated with peptide esters or amides (column 9, lines 59-61). Thus, '112 teaches away from administering LLME treated donor lymphocytes or co-administering CD34+ and treated CD34- fractions after hematopoietic stem cell transplantation, engraftment, or to a mammal requiring CD34+ stem cells, as claimed in independent claims 2, 3, 5, 12, 13 and 14 of the present application.

Therefore, Rosenfeld, Small, and '112, when combined, do not teach or suggest every element of the claimed invention, and thus cannot render the present invention *prima facie* obvious.

Furthermore, there would have been no motivation to combine Rosenfeld, Small, and '112. None of these references, either alone or combined, teaches treating lymphocytes with LLME prior to infusion following hematopoietic stem cell transplantation or engraftment, or the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, and infusing the cells to prevent GVHD. On the contrary, Rosenfeld teaches treating marrow with LLME. One of ordinary skill in the art would not have been motivated to combine Rosenfeld and '112, which teach LLME treatment of marrow, with Small, which teaches treating opportunistic infection with unmanipulated lymphocytes, because unmanipulated lymphocytes contain cytotoxic T lymphocytes and would in fact contribute to GVHD.

Neither Small nor Rosenfeld teach or suggest that infusing LLME treated cells after hematopoietic stem cell transplantation or engraftment induces or accelerates recovery of CD4+ cells. In fact, the prior art suggests that LLME treatment of donor lymphocytes and infusion following transplantation or engraftment of hematopoietic stem cells would not accelerate CD4+ recovery (Keever et al., 1989, Blood 73:1340-1350). There would have been no motivation to combine Small, Rosenfeld, or '112, because none of them suggests the requisite CD4+ increase needed to decrease the risk of GVHD.

There would have been no motivation to use LLME treated donor lymphocytes to ward off opportunistic infection. Examiner's assertion that the increase in CD4+ cells, and depletion of CD8+ and NK cells, would decrease the risk of GVHD and increase the ability to ward off opportunistic viral infection is ill-founded, because CD8+ cells are in fact required in the response to viral infection (Small, page 469, column 2). Small in fact teaches away from administering LLME treated donor lymphocytes or co-administering LLME treated CD34- cell fractions with CD34+ cell fractions to ward off opportunistic viral infection, because CD8+ cells are required to respond to viral infection. There would be no motivation to use LLME treated donor lymphocytes to ward off opportunistic infection because CD8+ cells would be depleted. Thus, there would be no motivation to combine Small with Rosenfeld and '112, because administering CD8+ depleted (LLME treated)

lymphocytes following a bone marrow transplant would be contrary to accepted wisdom regarding treating opportunistic infections and GVHD.

Furthermore, it would not be suggested from Small, or from the combination of Rosenfeld, Small, and '112, that infusion of LLME treated cells would restore protective immunity or inhibit GVHD. Even if CD4+ cell counts were to normalize following infusion of LLME treated cells, there is no prior art suggesting that viral infections will be controlled if CD8+ cells do not reconstitute. Neither Small, Rosenfeld, nor '112, either alone or combined, suggest that CD8+ cells will regenerate after LLME treated cells are infused. Thus, there would be no motivation to use LLME treated cells to treat or ward off viral infection, or to inhibit GVHD, from the combination of Small, Rosenfeld and '112.

Rosenfeld, Small, and '112, when combined, do not teach or suggest separating cells into CD34+ (stem cell) and CD34- (nonstem cell) fractions before LLME treatment, as claimed in claims 5 and 14. Nor do they teach treating only the CD34- fraction (comprising NK and cytotoxic T cells), while leaving the CD34+ fraction (CFU-GM containing) untreated, thus obtaining the benefits of NK and cytotoxic T cell reduction, without concurrent CFU-GM reduction. As described above, Rosenfeld and '112 teach administering bone marrow, while Small teaches administering unmanipulated and untreated lymphocytes to ward off opportunistic infections. Rosenfeld required administration of all LLME treated marrow cells. Independent claims 5 and 14 allow all of the CD34+ cells to be administered, while the dose of LLME treated CD34-cells can be controlled. In addition, the combination of Rosenfeld, Small, and '112 does not teach or suggest controlling the administration of different cell populations, thus allowing a dose of T cells to be administered which does not trigger GVHD. Thus, there would be no motivation to combine these references.

Therefore, because there would have been no motivation to combine Rosenfeld, Small, and U.S. Patent No. 5,668,112, and because the combination does not teach or suggest every element of independent claims 2, 3, 5, 12, 13, and 14, the combination of these references does not render *prima facie* obvious the invention as claimed. Thus, the rejection of independent claims 2, 3, and 5, as well as dependent claims 6, 8, 9, 10, and 11, under 35 U.S.C. § 103(a) should be withdrawn.

Response to the 35 U.S.C. § 112, first paragraph rejection

Claims 5-11 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description. In the interest of furthering prosecution, Applicants have canceled claim 7, without prejudice, to the filing of a continuation application. Therefore, the rejection as to this claim is now moot.

It is the view of the Examiner that the specification and claims as originally filed do not provide support for the phrases:

a) "obtaining a preparation of hematopoietic stem cells from a mammal," in claim 5;

b) "from about 10 micromolar to about 500 micromolar," in claim 8;

c) "at least about 500 micromolar," in claim 9; and

d) "for at least about 15 minutes," in claim 10.

Applicant respectfully points out that Examiner rejected claims 5-11 as allegedly lacking written description, but failed to provide reasons as to why claims 6 and 11 lack written description. However, Applicant will point out the written description for each of claims 5-11.

Although not necessarily agreeing with the reasoning of the Examiner, Applicant has amended claim 5, without prejudice, by removing the phrase "obtaining a preparation of hematopoietic stem cells from a mammal." Therefore, the rejection as to claim 5 is now moot.

The Examiner provided no reason for the rejection of claim 6. However, adequate written description is provided for claim 6 throughout the specification as filed, particularly at page 5, lines 11-20, page 8, line 11, page 9, lines 1-3, and in original claim 4.

Claim 7 is canceled herein without prejudice, therefore the rejection as this claim is moot.

Claim 8 has been amended to recite "from about 1 micromolar to about 250 micromolar." The amendment to claim 8 is supported at page 8, lines 27-29, of the specification as filed, and introduces no new subject matter.

Claim 9 has been amended to recite "375 micromolar." This amendment is supported at page 7, lines 9-12, and in FIGS. 1 and 2 of the specification as filed and introduces no new subject matter.

Claim 10 has been amended to recite "15 minutes." This amendment is supported at page 7, lines 9-12, of the specification as filed and introduces no new subject matter.

The Examiner provided no reason for the rejection of claim 11. However, adequate written description is provided for claim 11 throughout the specification as filed, particularly at page 8, lines 25-27.

Based on the amendments and reasoning described above, Applicants respectfully request withdrawal of the 35 U.S.C. § 112, first paragraph, written description rejection.

Conclusion

Based on the foregoing, all claims are believed to be in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,

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